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Research Article

A Novel Strategy for Donor Specific Antibody Detection for Haploidentical Hematopoietic Stem Cell Transplantation

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Abstract

Increasing numbers of haploidentical Hematopoietic stem cell transplants in Indian subcontinent has necessitated the use of more sensitive techniques for detection of donor specific anti HLA antibodies. Some centers that previously used only Complement Dependent Cytotoxicity Cross Match (CDCXM) have additionally started using Luminex Cross Match (LXM) alone or in combination with pooled bead assay. LXM and CDCXM were performed for 44 patients against their potential haploidentical 74 donors. Panel Reactive Antibody (PRA) screen was done for 37 patients. Donor specific antibodies were detected in 21 samples (28.4%) by LXM, PRA screen was positive in 17 /37 samples (46%) but CDCXM was negative for all patients. A combination of PRA screen with LXM was shown to be cost effective and useful strategy for detection of clinically significant donor specific antibodies in this study which would have been missed by CDCXM alone.

Keywords: Haploidentical Hematopoietic Stem cell transplant; Luminex cross match; Panel reactive antibody screen

Introduction

Bone Marrow Transplantation (BMT) is definitive therapy for both haematological malignancies and some non- malignant conditions such as Thalassemia and Sickle Cell Anaemia which are associated with significant morbidity and mortality. Haploidentical and less than fully matched BMT are often performed due to non-availability of HLA identical sibling, clinical urgency, low probability and high cost of getting a fully matched unrelated donor [1-4]. Presence of Donor Specific HLA Antibodies (DSA) of IgG isotype in Hematopoietic Stem Cell Transplantation (HSCT) is associated with significantly higher risk of failure to engraft [5-7]. The risk of DSA in HSCT has been recognized since over thirty years, but it is only in the last three years that they have assumed clinical significance in the Indian Subcontinent where Haploidentical transplants are preferred to cord blood transplants. PRA screen will detect all anti HLA IgG antibodies while DSA is expected to detect anti HLA antibodies directed against mismatched donor HLA AB and DR loci. This study pertains to the period when Luminex -X Map technology had been introduced in the country. Single Antigen Bead assay (SAB) had not been widely introduced, (commercial price $1000 per test) was six times as expensive as LXM, therefore not affordable for patients, most of whom meet the expenses themselves.

Materials and Methods

This study was carried out during the two- year period from July 2013 - December 2015 in the HLA Department of National Reference laboratory of Dr Lal Path Labs Ltd. which is accredited by The College of American Pathologists and the National Accreditation Board for Testing and Calibration Laboratories.

Patient and donor selection

None of the patients had a HLA matched related donor available so all available first-degree relatives and eight haploidentical extended family members were evaluated as possible donors. Table 1 depicts the primary disease for the patients, and the age /gender of patients and their prospective donors.

Samples

Whole blood from patients and donors were collected in Acid Citrate Dextrose (yellow top vacutainer) for isolating peripheral blood cells for CDCXM and LXM. Blood samples for serum was collected in red top vacutainers and blood for DNA isolation was collected in EDTA vacutainers all of which were procured from Beckton Dickinson (USA).

Immunogenetic work up

All assays except for CDCXM were done on LIFECODES products purchased from Immucor India Pvt. Ltd. and tested on Luminex 200. The procedure mentioned in the product was followed for all tests and both positive and negative controls were included in each run for all tests. Analysis of the accrued data was done by Quick Type Software initially and later by matchIT! After up gradation of the Software.

Low -intermediate resolution

Seven alleles DNA typing for HLA – ABCDRB1DRB345DQB1 was performed by reverse SSO on Luminex platform for 30 patients and their donors. Typing for the remaining samples was performed in the referring hospital itself or outsourced to Histogenetics USA.

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Table 1: Demographic, disease, sensitization and donor profile of 44 patients.

Recipients	(n = 44)
Children	16
Gender distribution (Recipient)	
Male	33 (75%)
DSA positive	9 (25.6%)
Females	11 (25%)
DSA positive	05 (44.5%)
Age (years)	
Range (Mean)	1.5 – 74 (27.3)
Primary Disease	
Acute Leukaemia-	25
Chronic Leukaemia	2
Aplastic Anaemia	6
Others	11
Donor profile	(n = 74)
Siblings	24
Parents	31
Progeny	11
Extended family	8
Age (years)	
Range (Mean)	1.5 – 60 (35.6)

PRA screen for HLA

Class I and II IgG antibodies weretested for 37 patients using LifeScreen Deluxe kits .Although it is essentially a screening test, we validated patient sera reactivity againstseven HLA- class I and five class II beads, and graded the reactions as weak, intermediate and strong based on the number of beads reacting and their Mean Fluorescence Intensity (MFI). This grading was validated in the laboratory against phenotype assay kits from the same vendor and had showed excellent correlation.

Luminex cross match

For donor specific HLA-Class I and II IgG antibodies was done for all 44 patients against 74 samples from 1-5 donors to select the most appropriate donor using LIFECODES Donor Specific Antibody Kits. The test was run in batches of 8-12 twice a week. The test procedure mentioned in product insert was followed– the principle of which is outlined below:

1. Donor lysate preparation from peripheral blood mononuclear cells separated by density gradient centrifugation.

2. Capture of solubilized donor HLA: Lysate 8µl and 5µl of beads are required for each test which are incubated in the dark for 30 minutes at ambient Room Temperature [RT] (20 -240 Celsius). This serves as a target for serum anti-HLA antibodies.

3. Incubation of donor -HLA and bead with serum diluted in specimen diluent for antigen -antibody reaction for thirty minutes in the dark at RT.

4. Labeling and Detection- Following another wash the diluted anti-Human IgG Phycoerythrin (PE) conjugate is added. After half an hour incubation at RT, a final wash is given and the plate is then placed in Luminex for acquisition of data and analysis.

5. Internal Quality Control was in form of a negative, a known positive control in all runs with lysate control in some runs.

6. Auto LXM was done for three samples from two recipients that gave unexpected class II positive DSA against related donors who were HLA identical for DRB1 and DQB1 loci at low resolution.

7. All LXM positive samples were confirmed by pooled bead assay. LXM is essentially a qualitative test with the results in terms of MFI for HLA – Class I and II IgG DSA. Reporting was done as positive or negative and further graded as weak (MFI 500-1000)/intermediate (MFI 1001 -2000)/strong (MFI >2000) for which cumulative positive MFI was considered.

B and T -cell CDCXM

For thirty patients was done by the extended NIH method [8]. The recipient sera were tested undiluted and in doubling dilutions up to 1:16 after DTT treatment. Auto-cross match was done for all samples.

A combination of Intravenous Immunoglobulin and plasmapheres is used in allo sensitized patients.

Results

Male patients outnumbered females, but DSA were more prevalent in females, (Table 1) as has also been reported by Lefell, et al [9]. Pooled bead assay was performed for 37 patients, of whom 17 were negative; HLA -class I, class II and both classes of antibodies were present in one, ten and nine samples respectively (data not shown). LXM HLA – Class I reactivity alone was detected in four samples, HLA -Class II in ten and both classes of antibodies in seven samples. Peak MFI for Class I was higher (19945 versus 12895 (Table 2)) which was also shown to correlate with extent of PRA screen reactivity in terms of number of beads and their MFI reactivity.

High background

Negative controls with MFI > 500 but a score of 0 or 1 affected the interpretation of LXM results due to High background (HBG). Seven samples had a HBG of which three were false positive and four were negative. On testing for PRA screen all these samples were negative and were considered negative for DSA.

False positive

Three samples from two patients gave a false positive DSA (class II -two and both -one). All were PRA negative and the recipient and donor had identical HLA -DRB1 alleles. On further testing both samples were PRA negative and were positive on auto-LXM. Luminex auto-cross match was performed on fresh samples from the patients that tested positive for Class II DSA.

Discussion

Economic constraints have contributed to widespread use of LXM along with pooled bead assay for detection of DSA in Indian subcontinent. Solid phase immunoassay has been used either alone or in lieu of far less sensitive CDC crossmatch for detection of donor specific antibodies in haploidentical HSCT. A high degree

Table 2: Luminex cross match positive patient – donor profile.

S No	Recipient age	Gender	Donor age	Relation	Class I	Class II	Grade
1	52	M	67	brother	289	856	weak
2	27	M	4	son	1941	518	strong
3	41	M	8	son	169	625	weak
			11	son	152	784	weak
4	40	F	37	sister	1178	1168	strong
5	45	M	40	Brother	3523	200	strong
6	2	F	31	mother	3164	755	strong
			30	father	1308	641	intermediate
7	45	M	61	sister	2179	497	strong
			37	nephew	19945	300	strong
8	27	M	31	sister	2164	873	strong
9	4	M	1.5	brother	247	1968	strong
			30	Father	477	1996	strong
10	33	M	35	sister	321	537	Weak
			59	Father	150	1692	intermediate
11	23	M	65	Father	406	2116	strong
			60	mother	200	4977	strong
12	30	F	34	sister	250	3563	strong
13	17	F	58	mother	3410	12895	strong
			50	father	1450	375	intermediate
14	1.5	F	22	uncle	733	1491	strong

of concordance was observed betweenpooled bead assay and LXM for detection of HLA –ABDR antibodies. It may therefore be logical to infer that a combination of these two tests may be used instead of Single antigen bead assay which has been reported to be positive in many healthy non- immunized males [9,10]. LXM has not been used much in Europe and North America but now two centres in UK are planning to evaluate this test (personal communication). The test has been used successfully for detection of DSA in solid organ recipients in India, but this is the first study where it has been used in haploidentical HSCT. It is quite likely that with matched HLA DRB1, DQB1 is also likely to be matched and the relevance of anti -HLA DPB1 antibodies in HSCT is yet to be proved. This is the first ever study regarding successful application of pooled bead assay in combination with LXM detection of most relevant HLA IgG DSA in mismatched HSCT.

Some of the confounding factors that we faced were HBG and auto- positivity. High background may be seen in up to 10% of samples including repeat samples from the same patient, maybe more against particular class of anti- HLA beads, may persist in all SPI for antibody detection/ specification and may be even specifically against a given batch of reagents. Some laboratories add bovine serum albumin to reduce high background whereas others use more expensive Seraclean. It was possible to reduce background for some samples by an additional wash after addition of the conjugate in LXM without affecting the control values. One sample gave false positive class II MFI 3087 (score 3), but class I was 4471 (score 1) and was negative for PRA screen. It is recommended that such samples be retested, and if the HBG persists confirm the positivity by performing an additional test such as PRA screen. We were able to identify two false positive reactions by performing auto LXM.

Ten patients (22.8%) received a haploidentical BMT of which one with positive LXM had poor engraftment but subsequently with immune suppression the DSA level declined and another HSCT was performed.In the three-year period between2013 - 2016, many transplant centers in India have had a successful transplant program for both solid organs and HSCT without using SAB assay.

Conclusion

LXM in combination with pooled bead assay is likely to be a useful for detection of anti HLA –ABDR DSA in haploidentical HSCT. The test needs to be evaluated in multiple centres on larger sample size and if resources permit a parallel testing with SAB assay to conclusively establish its role.

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